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HLA-DRB and HLA-DQA/HLA-DQB allele and haplotype frequencies in Iranian patients with aggressive periodontitis

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Background and Objective: Genetic backgrounds play a key role in susceptibility to and protection against a spectrum of periodontal diseases. Like other infectious diseases, the human leukocyte antigen (*HLA*) have been found to be associated with periodontitis. This study aimed to investigate differences in allele and haplotype frequencies of HLA class II antigens in a sample of Iranian patients with aggressive periodontitis compared with a healthy control group.

Material and Methods: Fifty unrelated patients with aggressive periodontitis and 130 healthy volunteers were enrolled in this study. HLA genotyping for HLA-DRB, HLA-DQA1 and HLA-DQB1 was performed using the PCR with sequence-specific primers. Allele and haplotype frequencies were compared across groups.

Results: The frequencies of HLA-DQA1*03:01, HLA-DQB1*03:02 and HLA-DQB1*03:05 alleles, as well as that of the HLA-DRB1*04:01 allele, were significantly higher in patients with aggressive periodontitis compared with control subjects ($p = 0.01$, $p = 0.04$, $p = 0.05$ and $p = 0.04$, respectively). In contrast, the frequency of the HLA-DQB1*0603 allele was significantly lower in patients with aggressive periodontitis compared with control subjects ($p = 0.006$; odds ratio = 0.20). With regard to haplotype association, a significantly higher frequency of two haplotypes – HLA-DRB1*04:01/HLA-DQA1*03:01/HLA-DQB1*03:02 and HLA-DRB1*16:01/HLA-DQA1*01:03/HLA-DQB1*05:01 – was observed in patients with aggressive periodontitis compared with healthy controls ($p = 0.01$, odds ratio = 2.56 and $p = 0.05$, odds ratio = 5.38, respectively).

Conclusion: These results provide additional evidence that class II HLA polymorphisms, particularly in the DQ locus, are associated with protection against and susceptibility to aggressive periodontitis.

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Periodontitis is a multifactorial disease that is influenced by both environmental and genetic factors (1, 2). A growing body of evidence attributes the interindividual differences in susceptibility to periodontal destruction to various immunologic and genetic factors in different populations (3–5). Segregation analyses have described

the familial occurrence of aggressive periodontitis via different Mendelian inheritance modes, as linkage analyses of chromosomal "hot spots" that provide certain families with genetic predisposition for aggressive periodontitis. (6, 7).

Genes involved in the manifestation of periodontitis and those involved in the immune process [including interleukin (IL)-1, IL-4, IL-6, tumor necrosis factor, IL-10, E-selectins, Fc-gamma receptor, CD14, toll-like receptors, N-formylpeptide receptor, caspase recruitment domain 15 and vitamin D receptor] have been reviewed in different studies (2, 3, 6, 8–11). As in other infectious diseases, and because of the effects on the antigen-specific T-cell response, the human leukocyte antigen (*HLA*) genes can be associated with periodontitis, and this area, especially *HLA* class I and II genes, has been of interest to researchers for many years (2, 12, 13). Numerous reports have disclosed correlations between HLA antigens of both classes and different types of periodontitis (6, 14–19). These etiologically distinct subtypes of periodontal pathogenesis would affect the therapeutic mode of disease, leading to individualized dental-treatment regimens based on pharmacogenetics (20). Recognizing susceptible subjects based on their genetic markers could lead to early-intervention strategies; this would be beneficial as aggressive periodontitis can be controlled more successfully in the early stages of progression than at later stages of the disease (21).

Owing to a lack of evidence related to genetic-risk determinants for periodontal status in middle-eastern populations, this study was conducted to establish the frequencies of HLA-DRB, HLA-DQA and HLA-DQB alleles in patients with aggressive periodontitis and to compare these frequencies with those found in healthy control subjects.

Patients and methods

This case–control study was performed, from June 2009 until May 2011, on 50 patients with aggressive periodontitis and on 130 periodontally healthy control subjects. The study was approved by the Research Ethics Committee of Tehran University of Medical Sciences. informed consent was obtained from all subjects. Exclusion criteria were: a history of chronic systemic diseases associated with periodontitis; being a current or a former smoker; a history of receiving corticosteroid therapy in the past 6 mo; and a history of receiving systemic antibiotic therapy in the past 6 mo.

Participants

Fifty patients with moderate to severe generalized aggressive periodontitis, referred to the department of periodontology, Tehran University of Medical Sciences, were selected as cases. The control subjects were selected from systemically healthy volunteers among the students and staff of Tehran University of Medical Sciences dental school. Each subject was examined using full-mouth probing and subjects with a mean probing depth of <3 mm and no proximal attachment loss were selected as controls. The controls were ethnically similar to the cases as both groups were selected from the Iranian populations. A routine blood examination was carried out to exclude the presence of any systemic disorder that may have contributed to the development of periodontal diseases.

Clinical examination

The subjects in both groups underwent a thorough periodontal examination, including measurement of the plaque index, the bleeding index and the clinical attachment level. The plaque index was evaluated according to Silness and Loe, and the bleeding index was assessed according to the National Institute of Dental and Craniofacial Research method (23). The clinical attachment level was measured using a Williams graduated periodontal probe at six sites around each tooth (22). To minimize interexaminer error, all measurements were recorded by the same examiner.

HLA genotyping

Peripheral blood samples from all study subjects were collected in ethylenediaminetetraacetic acid-containing tubes and then stored at −20°C until required for genomic DNA testing. Genomic DNA was extracted using the modified salting-out method described by Miller *et al.* (24). HLA typing was performed using the PCR with sequence-specific primers based on technical specifications provided by Olerup and Zetterquist (25). PCR kits with sequence-specific primers were purchased from Biotest (Heidelberg, Germany), and *Taq* DNA polymerase was purchased from Roche (Basel, Switzerland). Primers with intermediate resolution were employed for the amplification and typing of HLA-DRB, HLA-DQA and HLA-DQB alleles. The reaction mixtures were prepared in a final solution of 10 μl and amplified with an initial denaturation at 94°C for 2 min, followed by 10 cycles of denaturation (94°C for 15 s), annealing and extension (65°C for 60 s). The samples underwent an additional 20 cycles of denaturation (94°C for 10 s), annealing (61°C for 50 s) and extension (72°C for 30 s). The final PCR products were electrophoresed through a 2% agarose gel and then analyzed using an ultraviolet-light transilluminator. The specific and internal control bands were interpreted according to the kit manual and instructions. HLA-DR/DQ haplotypes were determined using the ARLEQUIN software (Arlequin Ver. 3.11 for windows, Bern, Switzerland) and also based on our knowledge and extensive experience of the linkage disequilibrium of HLA-DR and HLA-DQ alleles in the Iranian population.

Statistical analysis

The distribution of HLA alleles in case and control groups were compared using chi-square 2×2 contingency tables after Yates' correction for continuity. Fisher's exact test was used when necessary. In each comparison, the odds ratio (OR) along with the 95% confidence interval (95% CI)

was also calculated. All statistical analyses were performed using Epi Info version 3.3 for Windows (Centers for Disease Control and Prevention, Atlanta, GA, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results

Demographics and some clinical characteristics for the study subjects are summarized in Table 1. Allele frequencies of HLA-DRB1, HLA-DQA1 and HLA-DQB1 for patients with aggressive periodontitis and for healthy controls are depicted in Tables 2 and 3. There was an increased frequency of HLA-DRB1*04:01, and a tendency for a lower frequency of HLA-DRB1*13:01 in patients with aggressive periodontitis compared with healthy controls ($p = 0.04$ and $p = 0.09$, respectively; Table 2).

The frequency of HLA-DQB1*06:03 was significantly lower in patients with aggressive periodontitis compared with healthy controls ($p = 0.006$, OR = 0.20, 95% CI: 0.05–0.70; Table 3). On the other hand, patients with aggressive periodontitis had a significantly higher frequency of DQA1*03:01, DQB1*03:02 and DQB1*03:05 alleles compared with the control group ($p = 0.01$, OR = 2.56, 95% CI: 1.18–5.55; $p = 0.04$, OR = 2.17, 95% CI: 1.02–4.58; and $p = 0.05$, OR = 5.38, 95% CI: 0.83–42.96, respectively; Table 3).

We also observed slightly increased frequencies of HLA-DRB1*03:01 and HLA-DQA1*05:01 in patients with aggressive periodontitis compared with the control group, but the differences were not statistically significant (Tables 2 and 3).

With regard to the haplotype frequencies, we found that patients with aggressive periodontitis had significantly higher frequencies of two haplotypes – HLA-DRB1*04:01/HLA-DQA1*03:01/HLA-DQB1*03:02 and HLA-DRB1*16 :01/HLA-DQA1*01:03/HLA-DQB1*05: 01 – compared with the healthy control group ($p = 0.01$, OR = 2.56, 95% CI: 1.18–5.55; and $p = 0.05$, OR = 5.38, 95% CI: 0.83–42.96, respectively; Table 4). Additionally, the frequencies of two other haplotypes – HLA-DR B1*03/HLA-DQA1*05:01/H LA-DQ B1*02:01 and HLA-DRB1*15: 01/HLA-DQA1*01:02/HLA-DQB1*06:0 2 – were higher, although not statistically significant, in patients with aggressive periodontitis compared with the controls ($p = 0.07$, OR = 2.23, 95% CI: 0.94–5.28; and $p = 0.18$, OR = 2.23, 95% CI: 0.57–8.48, respectively; Table 4).

Discussion

While a large body of evidence exists regarding the association of various HLA polymorphisms with aggressive periodontitis in different ethnic groups, based on our knowledge these findings have not been replicated in the Iranian population (6, 13–19). In the current study, HLA-DQB1*06:03 was more frequent in healthy controls than in patients with aggressive periodontitis and therefore might have protective effects against periodontal disease. However, in accordance with our previous data for the Iranian healthy population we observed a higher frequency of HLA-DRB1* 15, *11 and *07, DQA1*05 and *01, DQB1*02 and DQB1* 05 alleles, and of HLA-DRB1*03/HLA-DQA1*0501/HLA-DQB1*0201, HLA-DRB1*07/HLA-DQA1*0201/HLA-DQB1*0201 and HLA-DRB1*11/HLA-DQA1*0505/HLA-DQB1*0301 haplotypes, in healthy controls in the current study (26–30).

Most studies on the association between HLA and aggressive periodontitis have investigated class I, but not class II, HLA molecules. Stein *et al*. conducted a meta-analysis of the studies carried out on the association between HLA and aggressive periodontitis (12). In this analysis, 42 HLA alleles (32 class I and 10 class II) were pooled, and nine studies met the inclusion criteria of the meta-analysis with all patients being of Caucasian ancestry (USA, Germany, Denmark, England and Israel) (12). The OR derived from this analysis revealed increased frequencies of A*09 and B*15, along with decreased frequencies of A*02 and B*05. a lower, but insignificant, frequency of HLA-DRB1*01 was observed among subjects with aggressive periodontitis (12). Similarly, the study of Shapira *et al*. showed a higher frequency of HLA-A*09 and *B15 alleles in patients with generalized periodontal involvement compared with patients with localized forms of periodontal disease (16). This association was also reported by Roshna *et al.,* on 40 patients with generalized aggressive periodontitis in whom HLA-B*15, but not HLA-A*09, was shown to be associated with aggressive periodontitis, and even with disease severity (31).

Machulla *et al*. investigated the difference in frequencies of HLA-DRB1 and HLA-DQB1 alleles between German subjects with aggressive periodontitis and healthy controls (19). Based on their observations, HLA-DRB*14 and HLA-Cw*08 were significantly increased in subjects with aggressive periodontitis compared with healthy controls, whereas a lower

Table 1. Demographics of the study subjects

Variables	Aggressive periodontitis group	Control group
Mean age (years)	38.06 ± 9.09	30.5 ± 7.77
Sex		
Female	67	61
Male	32	39
Familial history	79	25
Plaque index	1.85 ± 0.76	2.00 ± 0.54
Bleeding index	75	69
Clinical attachment level	4.43 ± 2.13	ND

Values are given as mean ± standard deviation or percentage.
ND, not determined.

Table 2. Human leukocyte antigen (HLA)-DRB1 allele frequencies in patients with aggressive periodontitis and healthy controls

HLA alleles	aggressive periodontitis group(n = 50)(100 alleles)		Healthy control group (n = 130)(260 alleles)		Odds ratio(95% confidence interval)	p-Value
	n	%	n	%		
DRB1						
01:01	6	6	17	6.5	0.91 (0.31–2.56)	0.95
01:02	0	0	2	0.8	na	ns
03:01	10	10	15	5.8	1.81 (0.73–4.48)	0.23
03:02	0	0	1	0.4	na	ns
04:01	16	16	21	8.07	2.17 (1.02–4.58)	**0.04**
07:01	17	17	33	12.7	1.41 (0.71–2.78)	0.73
08:01	2	2	5	1.9	1.04 (0.14–6.17)	0.70
09:01	0	0	1	0.4	na	ns
10:01	2	2	8	3.0	0.64 (0.09–3.34)	0.84
11:01	15	15	56	21.5	0.64 (0.33–1.25)	0.20
11:03	0	0	1	0.4	na	ns
12:01	0	0	2	0.8	na	ns
13:01	3	3	23	8.8	0.32 (0.07–1.15)	0.09
13:02	3	3	7	2.7	1.12 (0.22–4.92)	0.84
13:05	0	0	1	0.4	na	ns
14:01	6	6	14	5.4	1.12 (0.37–3.24)	0.97
14:02	0	0	1	0.4	na	ns
15:01	15	15	42	16.2	0.92 (0.46–1.81)	0.91
16:01	5	5	10	3.8	1.32 (0.38–4.33)	0.84

Significant difference was observed only for HLA-DRB1*04:01 allele; na, not applicable; ns, not significant.

Table 3. Human leukocyte antigen (HLA)-DQA1, and HLA-DQB1 allele frequencies in patients with aggressive periodontitis and in healthy controls

HLA alleles	aggressive periodontitis group(n = 50) (100 alleles)		Healthy control group(n = 130)(260 alleles)		Odds ratio(95% confidence interval)	p-Value
	n	%	n	%		
DQA1						
01:01	8	8	19	7.3	1.10 (0.43–2.78)	**1.0**
01:02	16	16	41	15.8	1.02 (0.52–1.99)	0.91
01:03	10	10	42	16.2	0.58 (0.26–1.26)	0.18
01:04	9	9	28	10.8	0.82 (0.34–1.90)	0.76
02:01	8	8	29	11.2	0.69 (0.28–1.66)	0.49
03:01	16	16	18	6.9	2.56 (1.18–5.55)	**0.01**
04:01	3	3	5	1.9	1.58 (0.29–2.74)	0.82
05:01	12	12	18	6.9	1.83 (0.79–4.2)	0.17
05:05	16	16	58	22.3	0.66 (0.34–1.27)	0.23
06:01	2	2	2	0.8	2.63 (0.26–26.53)	0.66[a]
DQB1						
02:01	20	20	44	16.9	1.23 (0.65–2.29)	0.59
03:01	18	18	61	23.5	0.72 (0.38–1.33)	0.32
03:02	16	16	21	8.07	2.17 (1.02–4.58)	**0.04**
03:05	4	4	2	0.8	5.38 (0.83–42.96)	**0.05**[a]
04:01	3	3	5	1.9	1.58 (0.29–7.74)	0.69[a]
05:01	14	14	43	16.5	0.82 (0.41–1.64)	0.66
05:02	7	7	17	6.5	1.08 (0.39–2.87)	0.93
06:01	8	8	19	7.3	1.24 (0.48–3.13)	0.79
06:02	5	5	6	2.3	2.23 (0.57–8.48)	0.18[a]
06:03	3	3	35	13.5	0.20 (0.05–0.70)	**0.006**
06:04	2	2	7	2.7	0.74 (0.10–3.96)	1.00[a]

[a]Two-tailed p-values, determined using fisher's exact test. Significant differences were found for HLA-DQA1*3:01, HLA-DQB1*03:02, HLA-DQB1*03:05 and HLADQB1*06:03 alleles.

Table 4. Most frequent human leukocyte antigen (HLA) class II haplotype in patients with aggressive periodontitis and in healthy control subjects

HLA-DRB1/HLA-DQA1/ HLA-DQB1 haplotypes	Aggressive periodontitis group (n = 50)(100 alleles)		Control group (n = 130)(260 alleles)		Odds ratio (95% confidence interval)	p-Value
	n	%	n	%		
01/01:01/05:01	5	5	16	6.15	0.80 (0.25–2.42)	0.86
03/05:01/02:01	12	12	15	5.7	2.23 (0.94–5.28)	0.07
04/03:01/03:02	16	16	18	6.92	2.56 (1.18–5.55)	**0.01**
07/02:01/02:01	8	8	29	11.15	0.69 (0.28–1.66)	0.49
10:01/01:04/05:01	2	2	8	3	1.75 (0.20–13.07)	0.62[a]
11/05:05/03:01	15	15	57	21.9	0.63 (0.32–1.22)	0.18
13:01/01:03/06:03	3	3	23	8.8	0.32 (0.07–1.15)	0.09
13:02/01:02/06:04	2	2	8	3	0.64 (0.09–3.34)	0.73[a]
14:01/01:04/05:02	6	6	14	5.4	1.17 (0.39–3.37)	0.96
15:01/01:02/05:01	2	2	11	4.23	0.46 (0.07–2.26)	0.52[a]
15:01/01:02/06:01	3	3	10	3.8	0.77 (0.17–3.13)	1.00[a]
15:01/01:03/06:01	3	3	9	3.46	0.86 (0.18–3.57)	1.00[a]
15:01/01:02/06:02	5	5	6	2.3	2.23 (0.57–8.48)	0.18[a]
16:01/01:03/05:01	4	4	2	0.7	5.38 (0.83–42.96)	**0.05**[a]
16:01/01:02/05:01	1	1	6	2.3	0.43 (0.02–3.64)	0.67[a]

alleles with a frequency of $\leq 1.0\%$ were not included in this table. Therefore, the total numbers of alleles in aggressive periodontitis and control groups were 87 and 232, respectively. Two haplotypes – HLA-DRB1*04 / DQA1*03:01 / DQB1*03:02 and HLA-DRB1*16:01 / DQA1*01:03 / DQB1*05:01 – were significantly more frequent in subjects with aggressive periodontitis than in controls.
[a]Two-tailed p-values, determined using fisher's exact test.

frequency of HLA-A*03 was noted in subjects with aggressive periodontitis. In comparison with control subjects, patients with rapidly progressive periodontitis had a significantly higher frequency of HLA- DRB1*13 (19). In contrast, we found a lower, but insignificant, frequency for DRB1*13:01 among patients with aggressive periodontitis compared with controls (p = 0.09). The difference in the ethnic backgrounds of the subjects in the two studies might account for the observed discrepancy.

In a survey of 24 Japanese patients with early-onset periodontitis, higher frequencies of DQB1*0503, DQB1*0602, DRB1*1401 and DRB1*1501 alleles were found, whereas healthy controls presented higher frequencies of DQB1*0401 and DRB1*0405 alleles (32). Similarly, Takashiba *et al*. showed an increased frequency of the HLA-DRB1*1501-DQB1*0602 genotype in patients with early-onset periodontitis (33). Another study, on European Caucasians with early-onset periodontitis, failed to replicate this pattern (34), whilst we observed significantly higher frequencies of HLA-DQA1*03:01, HLA-DQB1*03:02 and HLA-DQB1*03:05, as well as lower frequency of HLA-DQB1*06:03, in patients with aggressive periodontitis compared with healthy controls. It is noteworthy that, in contrast to the above study (32), our patients with aggressive periodontitis presented the HLA-DRB1*04:01 allele more frequently than did patients in the control group (p = 0.04).

When examining the role of HLA-DR4 on rapidly progressive periodontitis, Bonfil *et al*. (35) revealed that HLA-DR4*0401, HLA-DR4*0404, HLA-DR4*0405 and HLA-DR4*0408 are particularly more frequent in patients with rapidly progressive periodontitis. Likewise, an increased frequency of HLA-DR4 alleles has previously been reported in Israeli and Swiss patients (36,37). HLA-DR4 and HLA-A24 have also been linked to rapidly progressive periodontitis in a sample of 30 Turkish patients (18). These findings, in accordance with the results of our study, imply that the presence of the DR4 allele is more likely to be associated with aggressive periodontitis, which suggests a role for this allele as a risk factor in the development of aggressive periodontitis.

Additionally, herein we observed that HLA-DRB1*04:01/HLA-DQA1 *03:01 / HLA-DQB1*03:02 and HLA-DRB1*16:01/HLA-DQA1*01:03/ HLA-DQB1*05:01 haplotypes were significantly more frequent in patients with aggressive periodontitis compared with healthy controls. Consistently, the results of similar studies demonstrated an increased frequency for DR4 haplotypes (18, 35–38) and for DR2 (DRB1*15 and DRB1*16 alleles) haplotypes (37) in patients with rapidly progressive periodontitis and juvenile periodontitis, whilst Stien *et al*., on a study of the German population, revealed a higher frequency of two haplotypes – DRB1*04/DQB1*0302 and DRB1*15 – in patients with chronic periodontitis (15). Owing to the variety of HLA associations in different studies, it seems difficult to assign a single HLA marker to periodontal

diseases. Therefore, it is speculated that susceptibility/resistance to aggressive and chronic periodontitis may also be influenced by particular HLA marker combinations and other genetic factors (12, 15).

These inconsistent findings in different populations indicate that different HLA alleles may, directly or indirectly, affect the binding capacity of certain antigens to HLA molecules or other immune pathways, although with regard to the genetic background for periodontitis, associated HLA haplotypes may be of further importance for unknown gene loci (15). Nonetheless, further investigations of HLA haplotypes, especially on the presented HLA-DRB1 and HLA-DQB1 markers in relation to antigenic peptide-binding motifs, are necessary to verify the potential role of these HLA markers in periodontitis.

These preliminary results provide valuable insights on how host susceptibility might be involved in hindering or assisting the advancement of periodontal diseases. However, the rationale for a biological mechanism behind the HLA association with periodontal disease remains elusive and needs further studies in this regard.

It is noteworthy that the discrepancies observed between various studies could be caused, in part, by the influence of ethnicity and racial background on the distribution of HLA alleles. Moreover, differences in methodology, sample size and patient selection could also have served as a source of bias (11, 39) as some studies recruited a control group from blood donors with undetermined dental involvement, whereas in other studies (including the present study), healthy controls were included only after oral examination (18, 19).

Altogether, unlike autoimmune diseases (e.g. type 1 diabetes, where genome-wide association studies have denoted that classical HLA loci are at the frontier of genes that make patients susceptible to this disease), infectious diseases have not been firmly associated with HLA loci and reports declaring such an association have failed to publish conclusive results (40–42).

It is postulated that HLA molecules might be involved in initiating the inflammatory response via pathways other than those involving antigen presentation to immune cells (42). HLA-DR molecules expressed on human gingival fibroblasts are capable of inducing signaling pathways for the production of cytokines, including monocyte chemoattractant protein 1, IL-6, IL-8 and regulated on activation, normal T-cell expressed and secreted (RANTES) (42,43). These cytokines may have putative roles in sustained inflammation, which is characteristic of periodontal disease. Hence, defining a susceptibility profile that reflects the combined influence of the high-risk polymorphism (IL-1, IL-10, Fc-gamma receptor, tumor necrosis factor alpha, etc.) would be invaluable in therapeutic-intervention strategies aimed at preventing the development of periodontal disease (11, 39).

In conclusion, our study presents additional evidence on the role of HLA antigens in periodontal diseases in an Iranian sample. However, finding consistent patterns among HLA molecules that either confer or protect against aggressive periodontitis have yielded little success so far, partly because of significant variations in age, gender, ethnicity, racial background, diagnostic and clinical criteria, the number of cases and controls (which are often too low) and the patient-selection methods employed in association studies (11, 39). Nonetheless, further investigations, with larger and diverse populations, are required to clarify this association.

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